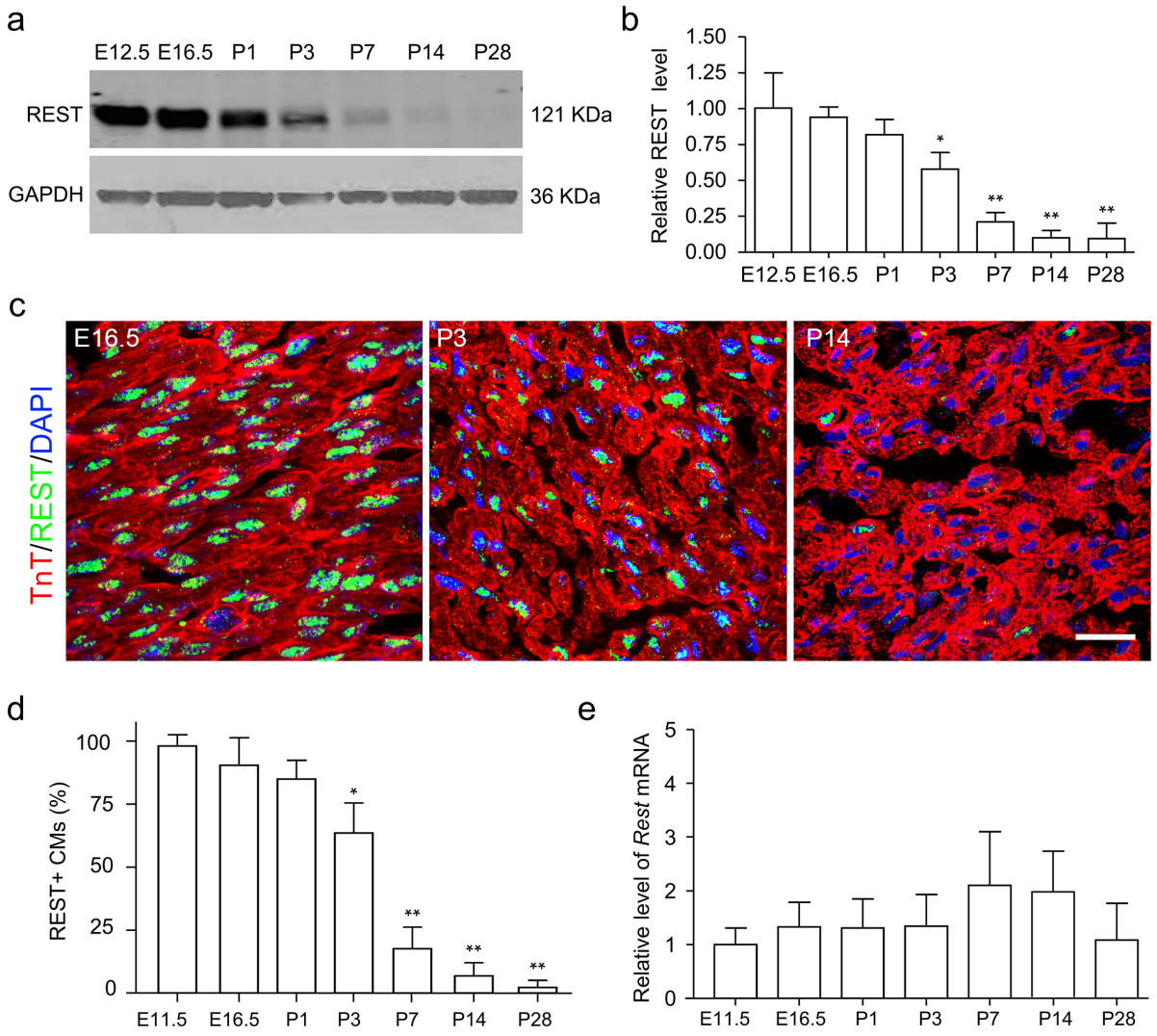
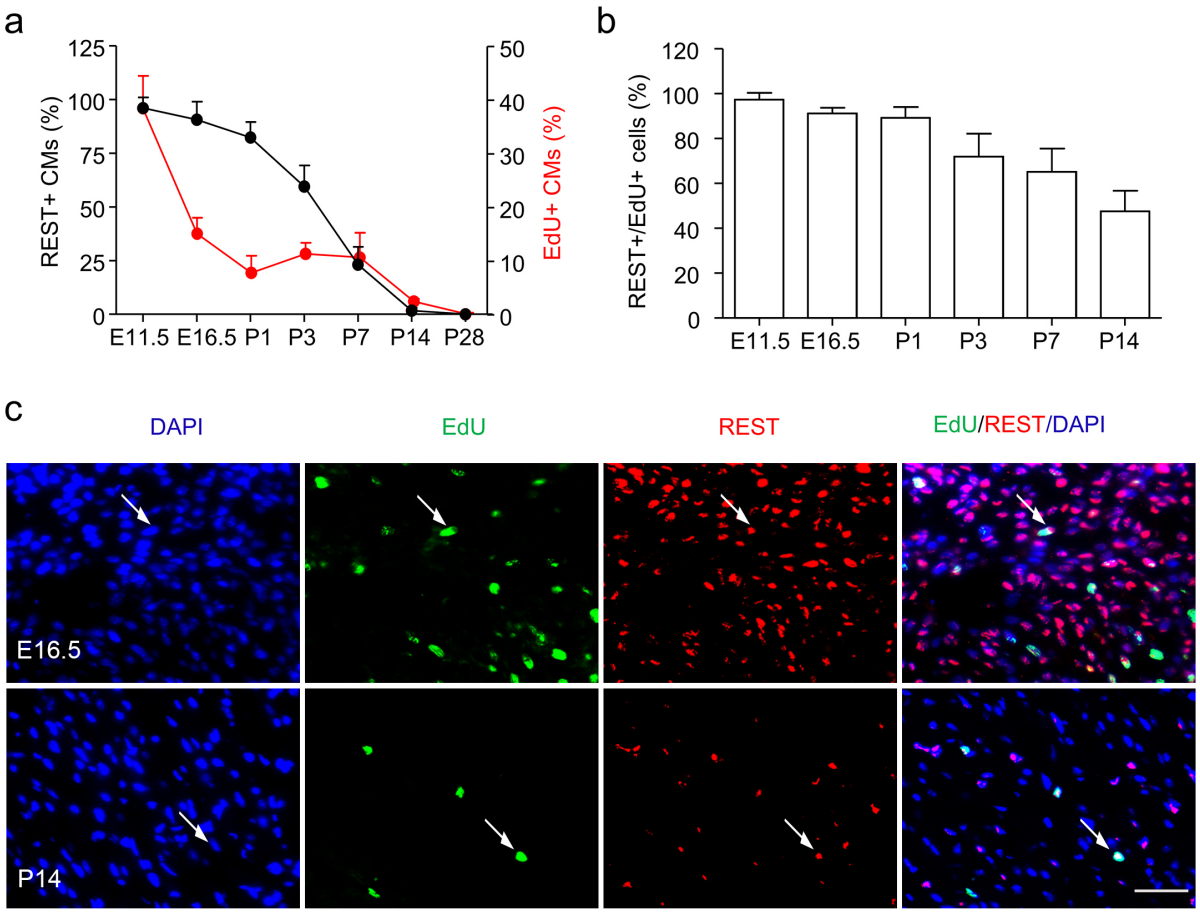
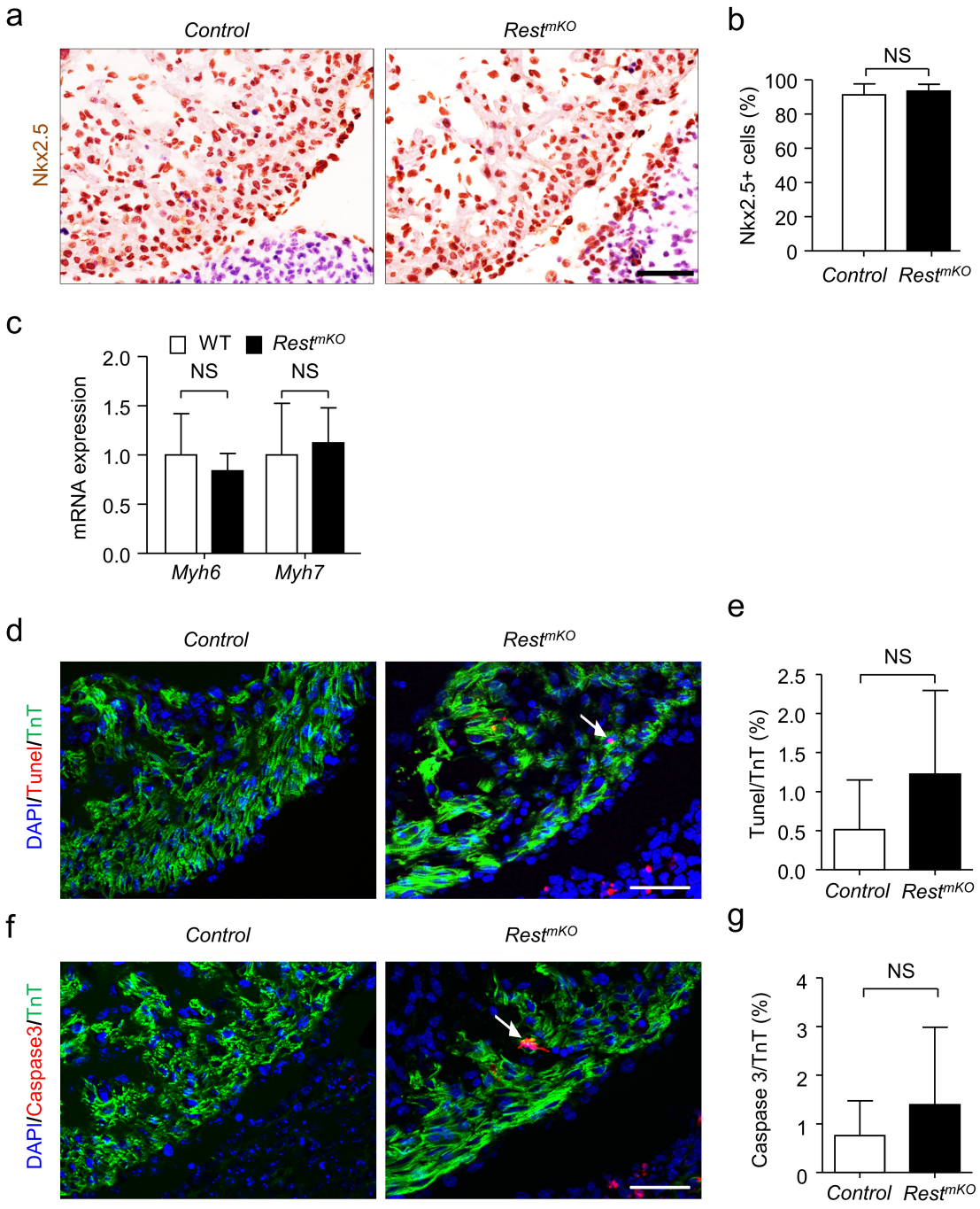


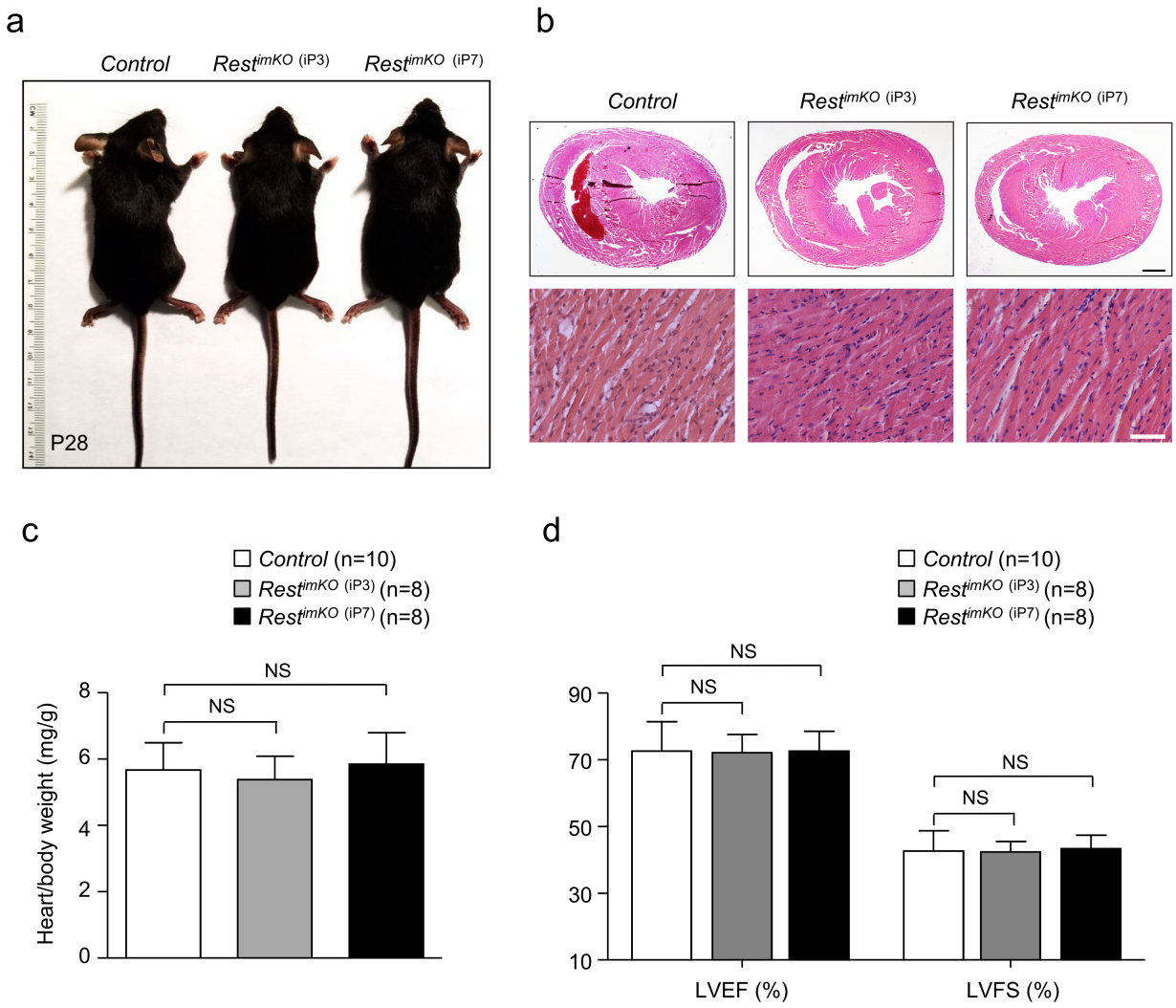
Supplementary Figure 1. *Rest* expression is developmentally downregulated in embryonic and postnatal hearts. a,**b**, Western blot analysis showing developmental downregulation of REST. **c**,**d**, Immunofluorescence showing REST expression in the majority of cardiomyocytes (CMs) of embryonic and neonatal hearts (from E11.5 to P3). After P3 the percentage of REST-expression cardiomyocytes is drastically decreased. Scale bar = 40µm. **e**, qRT-PCR indicating that *Rest* mRNA levels are sustained in the embryonic and postnatal hearts. E11.5 level as one, mean ± s.d. (n = 3/stage) in **a**,**b**,**e**, n = 6/stage in **c**,**d**. *$p < 0.05$, **$p < 0.01$ by one-way ANOVA followed by Tukey's test.



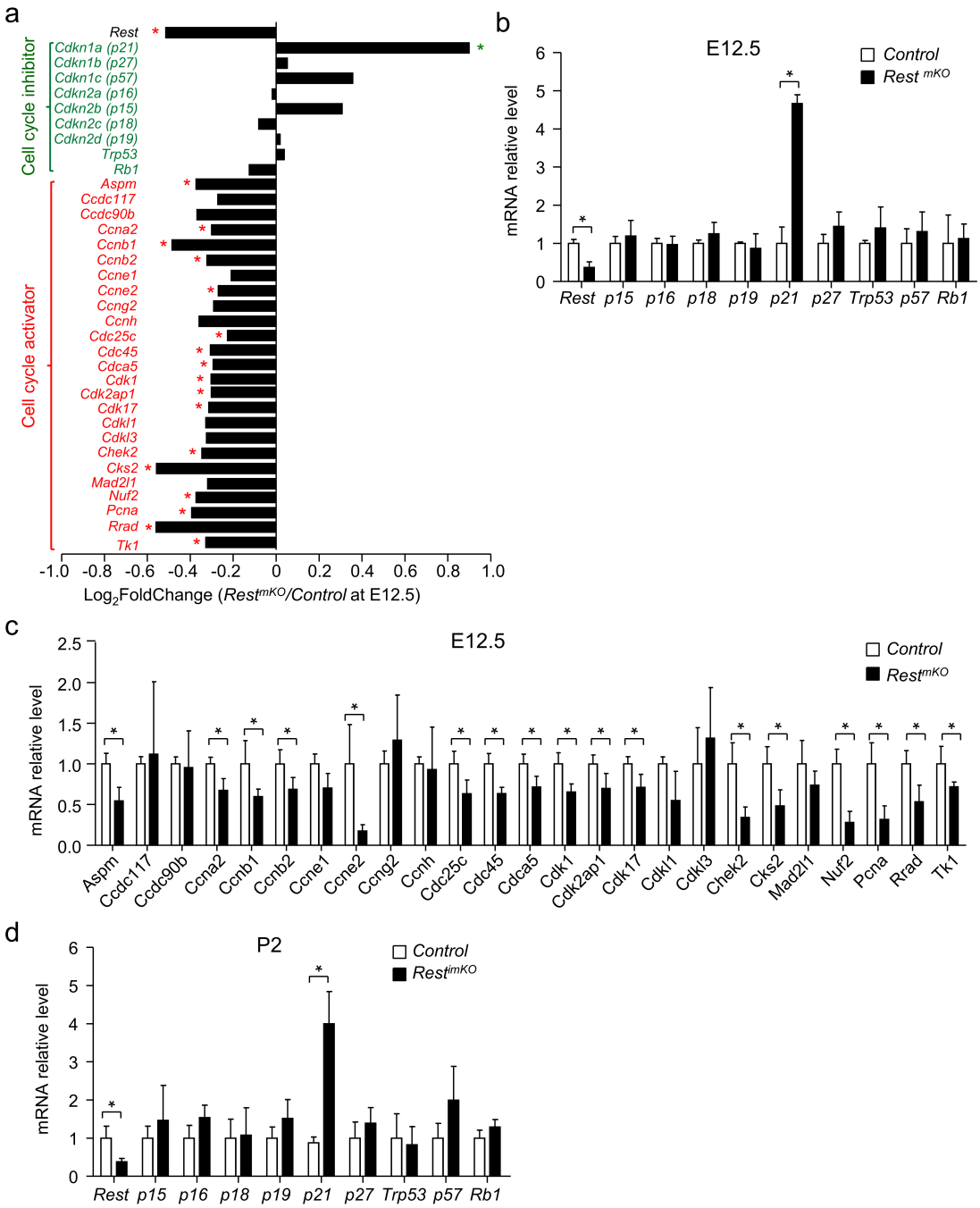
Supplementary Figure 2. REST expression positively correlates with cardiomyocyte proliferation. **a**, Graph showing developmental changes in the percentage of REST-expressing and EdU+ proliferating cardiomyocytes (CMs). **b**,**c**, Immunofluorescence revealing a majority of EdU+ proliferating cells expresses REST (arrow). Mean ± s.d., n = 6/stage for **a**; n = 4/stage for **b**,**c**. Scale bar = 40μm.



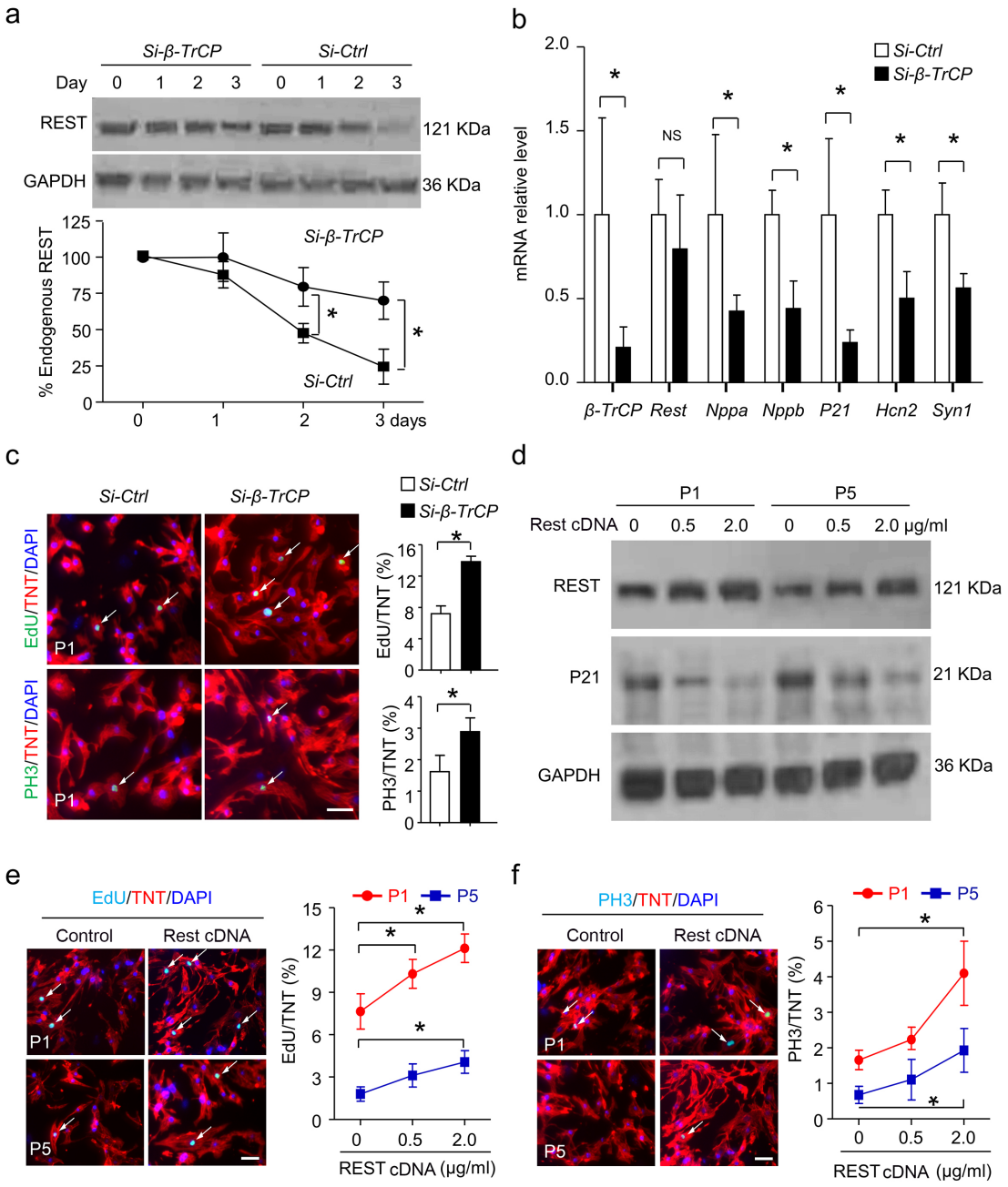
Supplementary Figure 3. *Rest* is not required for cardiomyocyte differentiation and apoptosis. **a**,**b**, Immunostaining showing no difference in the percentage of Nkx2.5-expressing cells in the ventricles between E12.5 control and *Rest*^{mKO} hearts. **c,** qRT-PCR analysis indicating no change in the level of *Myh6* and *Myh7* mRNA expression between E12.5 control and *Rest*^{mKO} ventricles. **d-g**, Immunofluorescence for TUNEL assay (**d**,**e**) and Cleaved Caspase 3 (**f**,**g**) showing no difference in the percentage of apoptotic cardiomyocytes (arrow) in the ventricles between E12.5 control and *Rest*^{mKO} hearts. n = 4/group for immunostaining, n = 3 for qRT-PCR, mean ± s.d. NS, no signification by unpaired two-tailed Student's *t*-test. Scale bar = 40μm.



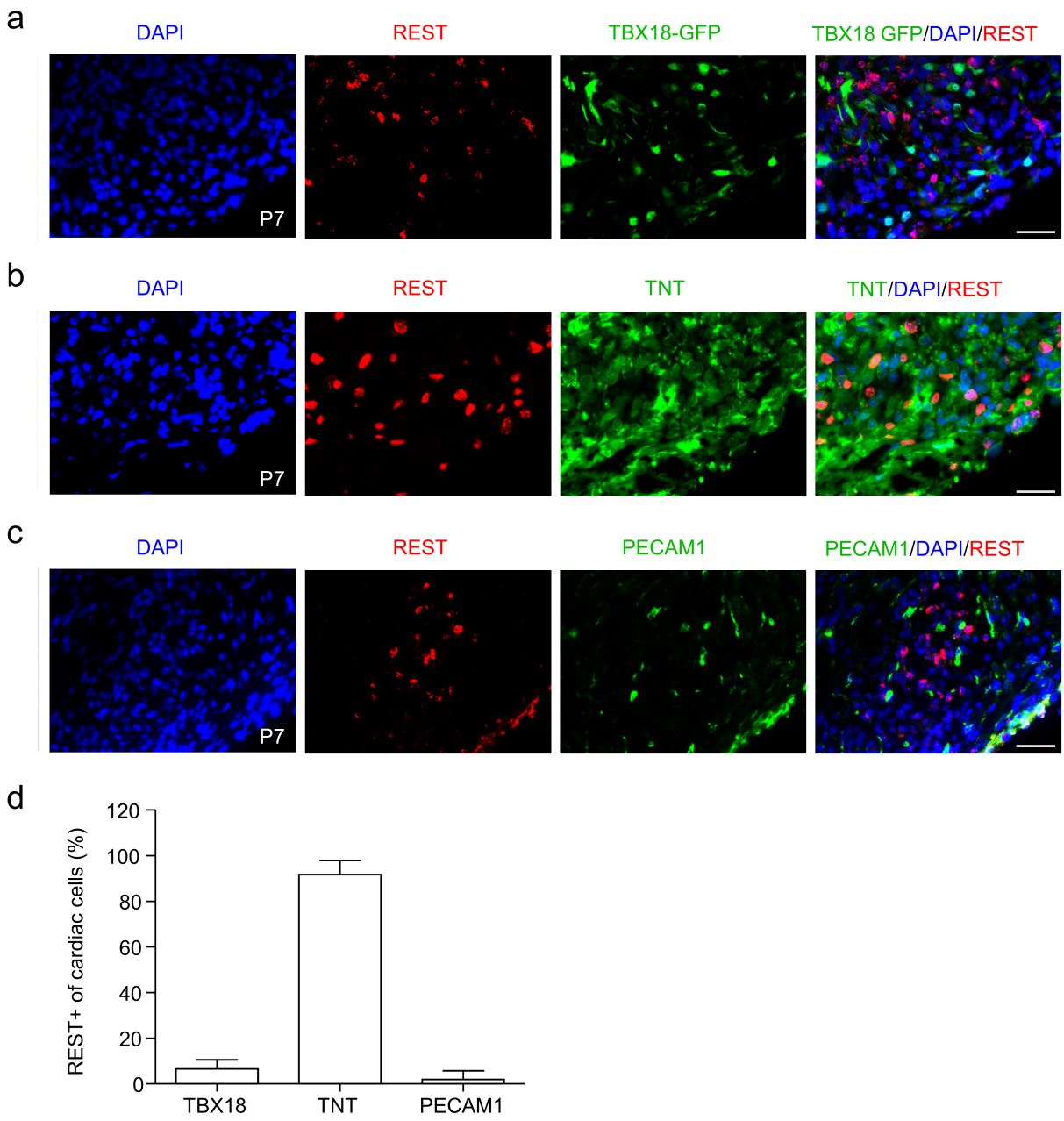
Supplementary Figure 4. Myocardial *Rest* deletion at P3 or P7 has no effect on cardiac structure and function. Normal appearance (**a**), cardiac structure by H&E staining (**b**), ratio of heart/body weight (**c**), and cardiac function on echocardiography (**d**) of P28 mice after *Rest* deletion induced at P3 or P7. Mean ± s.d. NS, no signification by one-way ANOVA followed by Tukey's test. Controls were Tam-treated $Rest^{+/+}$;$TnT^{MerCreMer/+}$ mice. Scale bar =100μm or 40μm in the top or bottom panel in **b**.



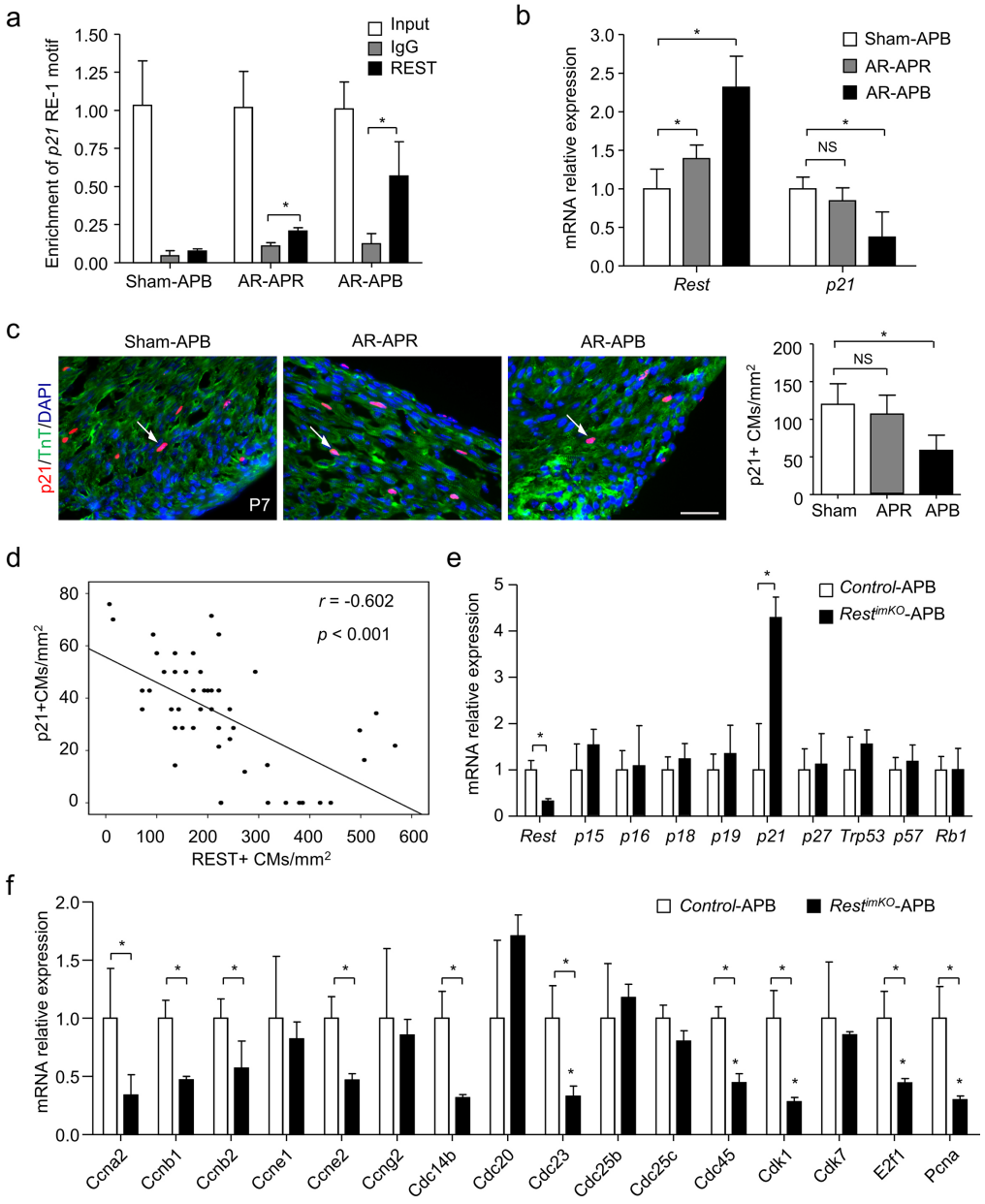
Supplementary Figure 5. *Rest* deletion results in dysregulated cell cycle gene expression. a, RNA-seq analysis of control versus *Rest* inactivated ventricles of E12.5 hearts showing that among the cell cycle inhibitor genes (green*), *p21* expression is upregulated, whereas the expression of several cell cycle activator genes is downregulation (red*). **b**,**c**, qRT-PCR shows *p21* upregulation (**b**) and downregulation of the cell cycle activator genes (**c**) in E12.5 ventricles with *Rest* inactivation. **d**, qRT-PCR shows *p21* upregulation in P2 ventricles with *Rest* inactivation at P1. Controls were the Tam-treated $Rest^{+/+}$;$TnT^{MerCreMer/+}$ mice. n = 3/group, mean ± s.d. *$p < 0.05$ by unpaired two-tailed Student's *t*-test.



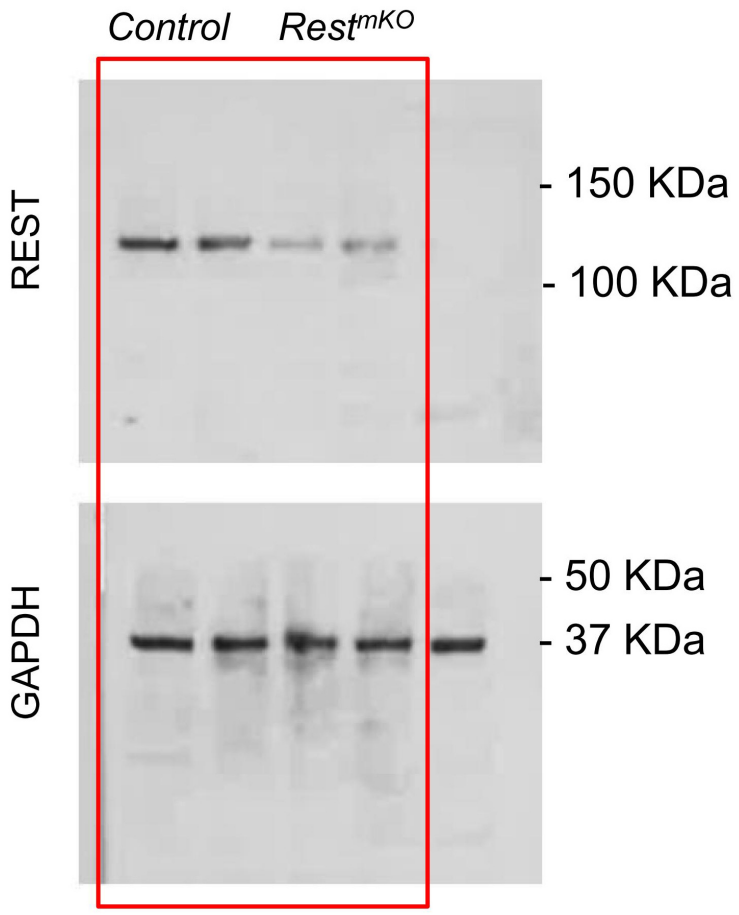
Supplementary Figure 6. Overexpression of REST induces cardiomyocyte proliferation *in vitro*. **a-c,** Transfecting siRNA against *β-TrCP* into the primary cultured cardiomyocytes isolated from P1 hearts stabilizes REST protein (**a**, western blot), downregulates the expression REST target genes (**b**, qRT-PCR), and promotes EdU incorporation and PH3 expression (**c**, immunostaining). **d-f**, Transfecting a plasmid containing Rest cDNA (pHR-NRSF-CITE-GFP, Addgene) into the primary cultured cardiomyocytes isolated from P1 or P5 hearts results in overexpression of REST with reduced p21 expression (d, western blot), increased EdU incorporation (**e**) and PH3 expression (**f**). Noted that GFP was not detectable in the assay. Data are presented as mean ± s.d. (n = 3).*$p < 0.05$ by unpaired two-tailed Student's *t* test in **a-c**; one-way ANOVA followed by Tukey's test in **e**,**f**. Scale bar = 40μm.



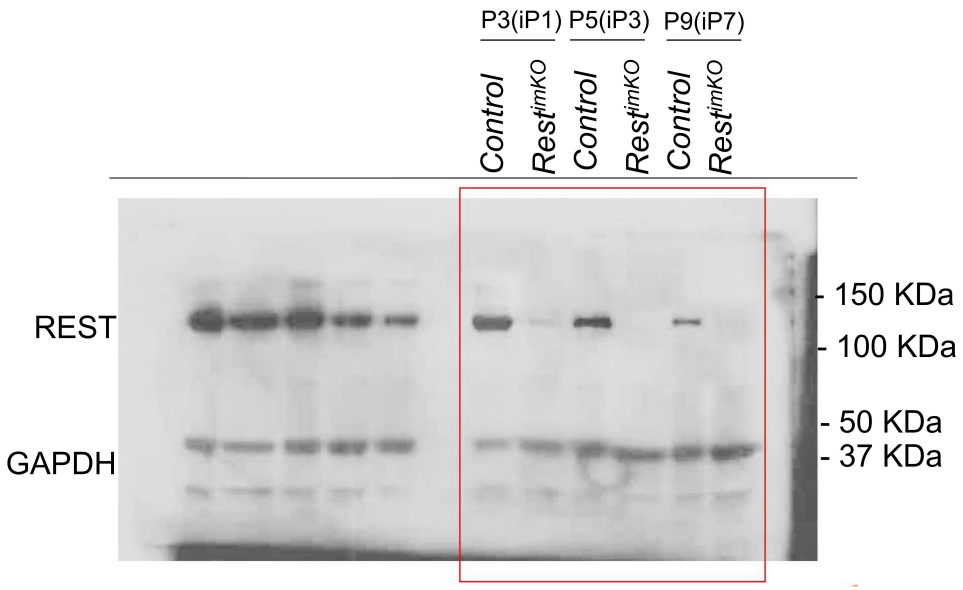
Supplementary Figure 7. REST re-expresses in cardiomyocytes in regenerating neonatal hearts. a-d, Quantitative immunofluorescence showing the majority (~90%) of REST re-expression cells are cardiomyocytes in P7 old regenerating hearts after apex ablation at P4, while a small percentage (<10%) of cells are epicardial or endocardial/endothelial cells. Scale bar = 40μm. n = 4/group, mean ± s.d.



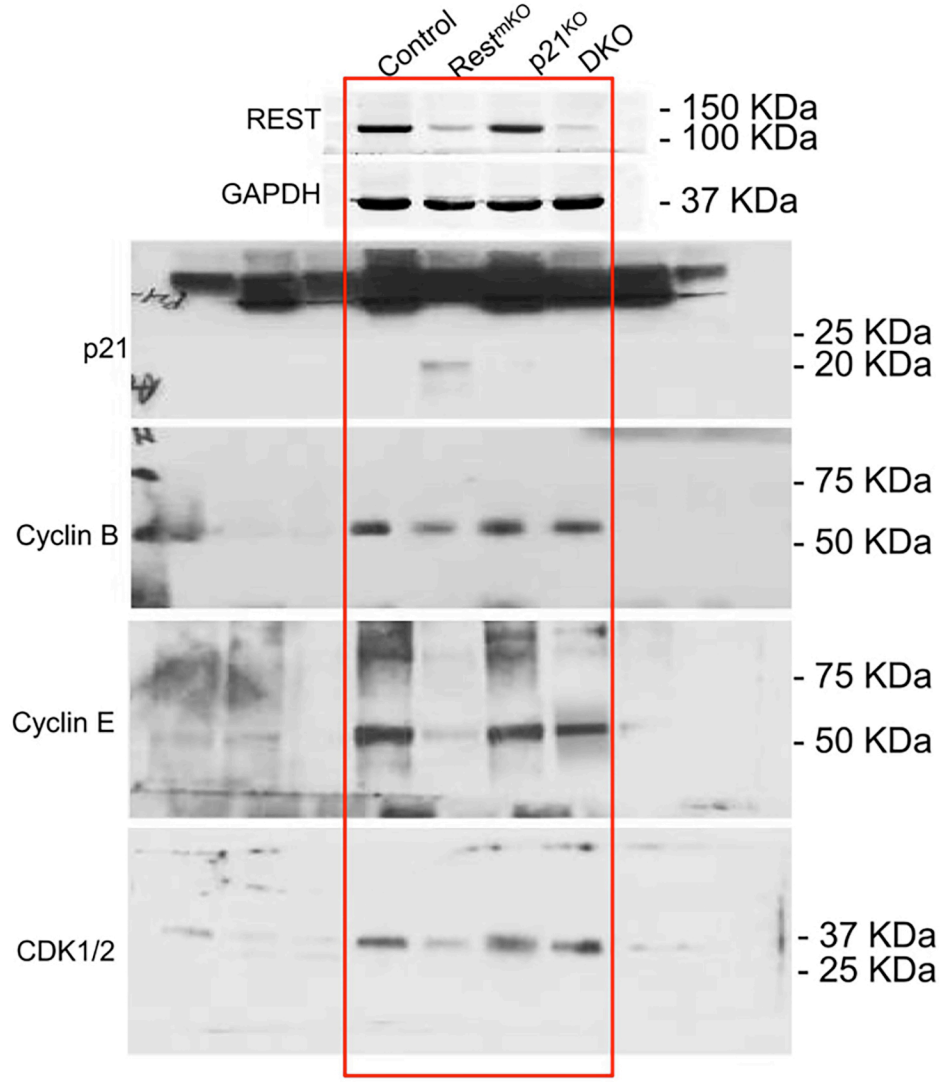
Supplementary Figure 8. REST represses *p21* expression in regenerating myocardium. a, qChIP indicating increased REST binding to the *p21*-RE1 motif containing DNA fragment in the regenerating apex of P7 hearts. **b**, qRT-PCR showing *Rest* upregulation and *p21* downregulation in the regenerating apex of P7 hearts. **c**, Immunofluorescence revealing decreased number of p21+ cardiomyocytes (CMs) in the regenerative apex of P7 heart, n = 4/group. Scale bar = 40μm. **d**, Scatter plots showing a negative relationship of the number of REST+ and p21+ cardiomyocytes. Average counts from 6 fields/section, 3-4 sections/ventricle, 4 ventricles/stage at P7, 14, and 28. **e**,**f**, qRT-PCR analysis revealing changes in the levels of cell cycle inhibitors (**e**) and activators (**f**) in the regenerating apex resulting from *Rest* deletion. n = 3/group, mean ± s.d. **p*<0.05 by one-way ANOVA followed by Tukey's test in **a**-**c**; unpaired two-tailed Student's *t* test in **e**,**f**. APB/APR, apex border/apex remote.



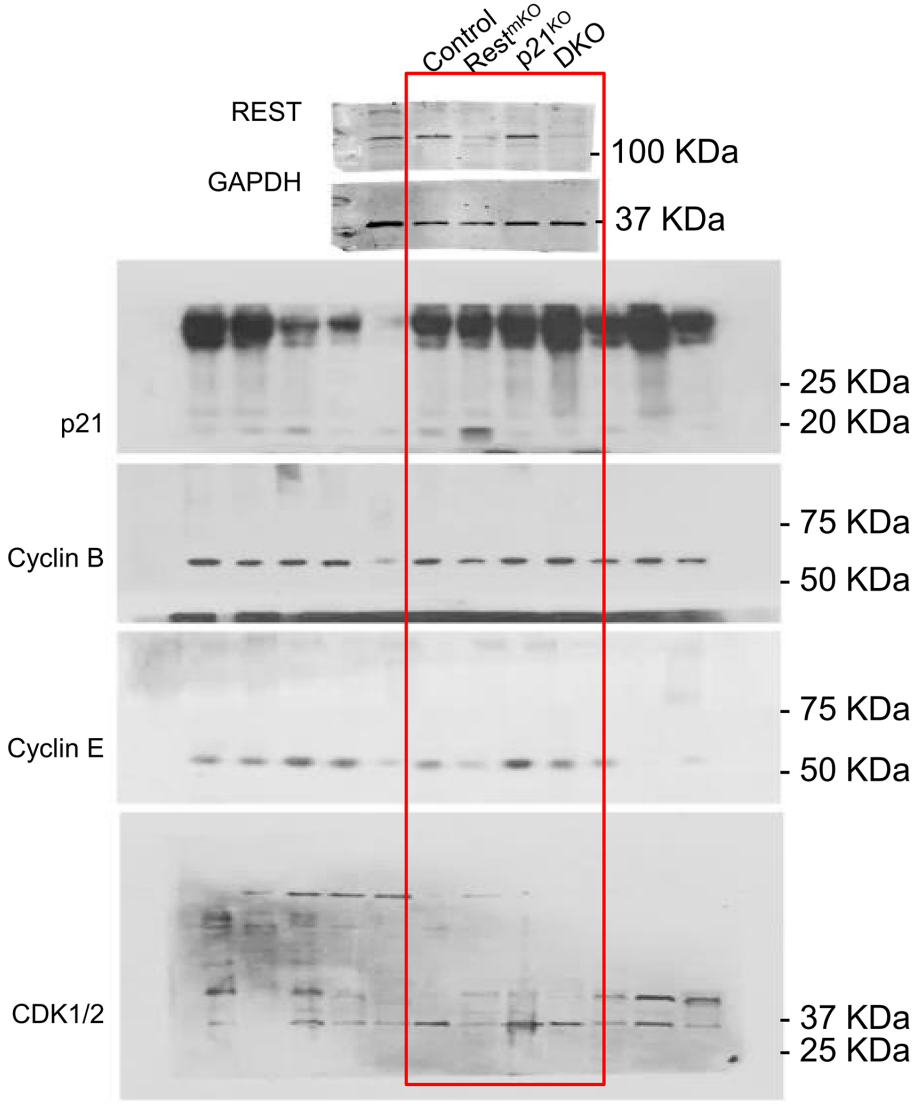
Supplementary Figure 9. Full scan of western blots shown in Fig. 1b.



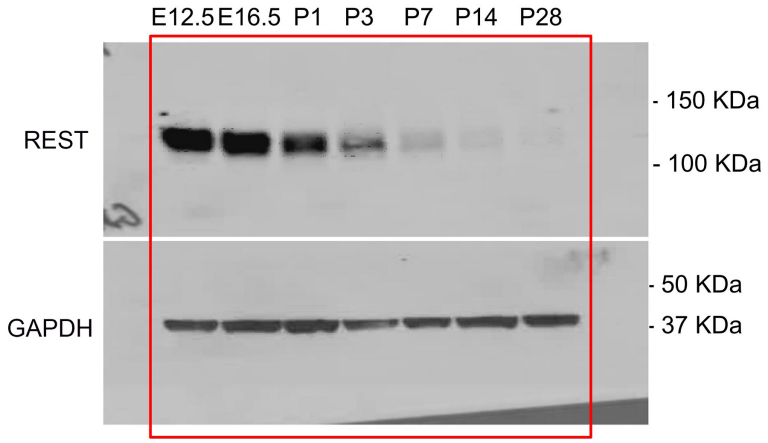
Supplementary Figure 10. Full scan of western blots shown in Fig. 2b.



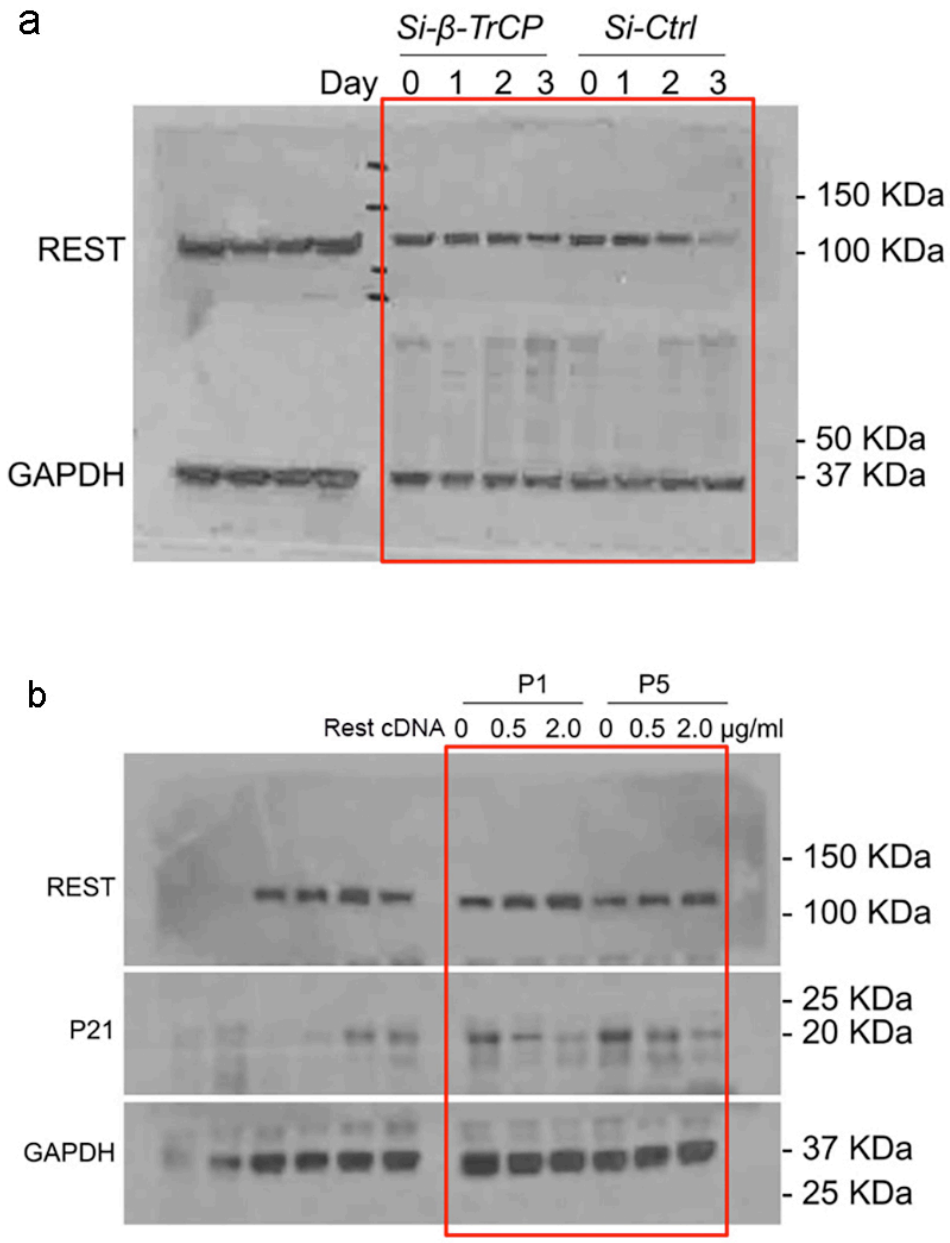
Supplementary Figure 11. Full scan of western blots shown in Fig. 4d.



Supplementary Figure 12. Full scan of western blots shown in Fig. 5f.



Supplementary Figure 13. Full scan of western blots shown in Supplementary Fig. 1a.



Supplementary Figure 14. Full scan of western blots shown in Supplementary Fig. 6a (**a**) and 6d **(b)**

Supplementary Table 1. List of primers used in qRT-PCR, qChIP and luciferase reporter assays

Assay	Primer sequence (sense 5'-3')	Primer sequence (anti-sense 5'-3')
qRT-PCR		
Aspm	TGGCTATGAGTGAATGCTCTTCC	TCGCGTAAAAACAGTGGCAAG
Ccdc117	GGCTGCAAGAGATTGAGGACA	CCATAGAAGGACGGCTCATAGAC
Ccdc90B	TCCGCTGGACTTCAACTCCT	ACCAACGCATGGGTATCAAAAG
Ccna2	AAGAGAATGTCAACCCCGAAAAA	ACCCGTCGAGTCTTGAGCTT
Ccnb1	CAATTATCGGAAGTGTCGGATCA	CTGGTGAACGACTGAACTCCC
Ccnb2	GCCAAGAGCCATGTGACTATC	CAGAGCTGGTACTTTGGTGTTC
Ccne1	GAAAAGCGAGGATAGCAGTCAG	CCCAATTCAAGACGGGAAGTG
Ccne2	ATGTCAAGACGCAGCCGTTTA	GCTGATTCCTCCAGACAGTACA
Ccng2	AGGCTACCCCGGAGAATGATA	ACCTTCATAAGGGCCAAGAATCT
Ccnh	CAGAAACGGCACTGGACCTT	GACCGTGGCATAGCTGGTT
Cdc14b	CTCTACAGCAGACCAAAGAGTG	CCTCAGCATTGTAATGGACTTGA
Cdc20	TTCGTGTTCGAGAGCGATTTG	ACCTTGGAACTAGATTTGCCAG
Cdc23	TTCTTCGGTAGTCTCGGTGG	CCAACTCCGCAGACCATTT
Cdc25b	TCCGATCCTTACCAGTGAGG	GGGCAGAGCTGGAATGAGG
Cdc25c	GTTTCAGCACCCAGTTTTAGGT	AGAATGCTTAGGTTTGCCGAG
Cdc45	TCAGAGGCTGAATGGGTAACA	GGGAAGAACTATGAGGTCTGGT
Cdca5	CGGCGAACACGATCAGGAG	TCCTCGGCCAGATTTCAGAGA
Cdk1	TCAAGCACCTGGACACAATTC	GGACCCTTTTGACTTGGTTGG
Cdk17	AACCTTGCACGACATCGTTCA	CACGGAGAATTTGGTACAGGAA
Cdk2ap1	GCGACCTCCTCCCAATATCG	GTCTGATCTCTTTCCCCAACTCT
Cdk7	GACACCATCCCACATTAAAGCC	CACCATACATCCTAGCTCCAAAC
Cdkl1	AGAAGGCTCCTATGGGGTAGT	GGCGATTTTCTTTATGACAGGGT
Cdkl3	TAAGGACACTGGGCGAATAGT	GACCGTGTGGTCAATAAACTCAA
Cdkn1a	GAAAGAAGCGGAAGATCCTCC	GGGCCTCAGGGATTGTTTGG
Cdkn1b	TCAAACGTGAGAGTGTCTAACG	CCGGGCCGAAGAGATTTCTG
Cdkn1c	GCAGGACGAGAATCAAGAGCA	GCTTGGCGAAGAAGTCGTT
Cdkn2a	CAAAGTGACAGATGCTCCAATCC	TTTTCCTTCTACGGCTCGTTTT
Cdkn2b	CCCTGCCACCCTTACCAGA	CAGATACCTCGCAATGTCACG
Cdkn2c	GGGGACCTAGAGCAACTTACT	AAATTGGGATTAGCACCTCTGAG
Cdkn2d	CTGAACCGCTTTGGCAAGAC	GCCCTCTCTTATCGCCAGAT
Chek2	TGACAGTGCTTCCTGTTCACA	AGCTGGACGAACCCTGATACT

Cks2	TCGATGAGCACTACGAGTACC	CCATCCTAGACTCTGTTGGACAC
E2f1	TGCAGAAACGGCGCATCTAT	CCGCTTACCAATCCCCACC
Gapdh	ACGGCAAATTCAACGGCACAGTCA	TGGGGGCATCGGCAGAAGG
Mad2l1	TCCGTCGGAAACCTTTACTAGA	ACTGCCATCTTTCAAGGACTTC
Nppa	ATTGACAGGATTGGAGCCCAGAGT	TGACACACCACAAGGGCTTAGGA
Nppb	AGGGATACAGGAGCTGCTGG	CCTCGCCTCCCAGGCTTCTG
Nuf2	TCCCCAGATACAATGTAGCTGA	CCGGACTCCATACACTAACTGT
Pcna	TTGCACGTATATGCCGAGACC	GGTGAACAGGCTCATTCATCTCT
Rest	GTGCGAACTCACACAGGAGA	GAGGTTTAGGCCCGTTGTGA
Rb1	GGAACCCAACTCAACGTGAC	TCTGGTCGCTTGTGTGCTC
Rrad	GTCAGAGGAGGGCGTTTACAA	TCCACAGTGATAGAACGGTCA
Syn1	AATATGCGGCTATGGATGTTGG	TCCTGAGTGACCGGAGAGTC
BTRC	AAGACTGTAATAATGGCGAACCC	TCTCTTGGTTTATGCAAAGCCTG
qCHIP		
P21-RE1	CACCCCATACTTCCCCTTCT	AAGACCAGGGAATCCCACTT
Luciferase assay		
P21-RE1 WT	CGTGGGTACCCACTTCTTACCTGGGGGTGA	AATGGCTAGCCCATTGCTCAGTGTCCTGTG
P21-RE1 Mut1	CTAGGCACTTGCCCCAAAAAACAGTGGACTGG	CCAGTCCACTGAGCTGTGGGGTTTTTGCCTAG
P21-RE1 Mut2	CCACAGCTCAGTAAAAAAGAAGGGAATGTATATG	CATATACATTCCCTTCTTTTTTACTGAGCTGTGG
P21-RE1 Del	CTAGGCACTTGCCCCAGAA GGGAATGTATATGC	GCATATACATTCCCTTCTGGGGCAAGTGCCTAG